(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 July 2002 (11.07.2002)

PCT

(10) International Publication Number
WO 02/053166 A1

(51) International Patent Classification[7]: A61K 35/78

(21) International Application Number: PCT/IN01/00225

(22) International Filing Date:
27 December 2001 (27.12.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/258,848 28 December 2000 (28.12.2000) US
60/258,849 28 December 2000 (28.12.2000) US

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *with amended claims and statement*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/053166 A1

(54) Title: USE OF TINOSPORA EXTRACT IN THE TREATMENT OF IMMUNE SYSTEM-MODULATED DISORDERS

(57) Abstract: Immunomodulating pharmacologically and biologically active compositions containing a standardized extract of the plant *Tinospora cordifolia*, a process for their preparation and their use in dosage forms for healthcare, nutrition and treatment of disorders modulated by the immune system are described. The process includes preparation of an extract from plant parts of *T. cordifolia* and standardization of the extract by phagocytosis and LC-MS single ion recording assays. Methods of treating immunomodulatory deficiencies in healthcare are also disclosed using the compositions.

USE OF TINOSPORA EXTRACT IN THE TREATMENT OF INMUNE SYSTEM-MODULATED DISORDERS

Field of the Invention

The present invention relates to a novel method of treatment of health conditions associated with alteration or modulation of immunity. The present invention also pertains to a standardized extract of the plant *Tinospora cordifolia*, compositions containing the extract and use of the extract to treat health conditions and for treatment of disorders modulated by the immune system.

Background of the Invention

The immune system of an organ acts as a defense mechanism regulated by an intricate system of humoral and cellular factors. Both humoral immune and cell-mediated immune mechanisms operate together on one hand to eliminate foreign bodies such as pathogenic microorganisms or neoplastic cells, and on the other to prevent the rejection of organ and tissue transplants. The immune system becomes deficient or compromised due to several reasons, namely genetic, debility, age, infections, cancer, auto-immune mechanisms, and in recent years the acquisition of the immune deficiency syndrome (AIDS).

Immunocompromised conditions may be found in patients with the following infections, diseases, or disorders:

Ear, nose or throat (ENT) Infections: Chronic recurrent tonsillitis, Pharyngitis, Chronic otitis media, Peritonsillar abscess;
Respiratory system disorders: Tuberculosis, Chronic bronchitis, Chronic recurrent allergic bronchial asthma;
Gastrointestinal disorders: Recurrent Diarrhoea & Dysentery, Peritonitis, post-surgical abdominal infections;
Infections in immunocompromised host: Opportunistic infections in diabetes, Opportunistic infections in burns, opportunistic infections in malignancy;
Hepatobiliary diseases: Hepatitis, Cirrhosis of the liver, Obstructive jaundice,
Neutropenic patients: Patients on cancer chemotherapy

Autoimmune diseases: destruction of pancreatic beta cells leading to Insulin Dependent Diabetic Mellitus (IDDM) and
Surgical prophylaxis.

Another disease related to immunity is osteomyelitis. Osteomyelitis is an infection of bone and is caused most commonly by pyogenic bacteria and mycobacteria. Microorganisms enter bone through several ways, by the hematogenous route, by direct introduction from a contiguous focus of infection, or by a penetrating wound. They can bind to exposed sites of bone in which the susceptibility is enhanced by a variety of factors. The pathology of osteomyelitis is characterised by phenomena such as pus formation, lysed bone, devascularized bone fragments, subperiosteal or soft tissue abscesses, and in chronic cases, necrotic bone. Attendant symptoms are discharge, itching, odour, pain, tenderness and edema.

Especially in its chronic form, osteomyelitis is difficult to treat.

The treatment for osteomyelitis is based on classification of the disease, whether acute hematogenous, or vertebral, or secondary to a contiguous focus of infection, without or with vascular disease, and chronic forms of all such mentioned classes. Although current therapy reflects increased appreciation of the combined roles of antimicrobial courses and surgical debridement, the results especially in patients with chronic osteomyelitis are quite often discouraging.

Both approaches of antibiotic therapy and surgery are fraught with many limitations:

1) **the usual need for initial intravenous administration of antibiotics; few data support the use of oral antibiotic by adults except in the case of fluoroquinolones; also the high dose of oral penicillins or cephalosporins recommended are not tolerated well by adults.**
2) **toxicity associated with the use of aminoglycosides like gentamicin and tobramycin, especially in cases of osteomyelitis due to *Pseudomonas aeruginosa* or Enterobacter sp.**
3) **prolonged courses of antimicrobial therapy, especially in chronic osteomyelitis.**
4) **multiple surgical procedures.**
5) **intraoperative difficulties to determine whether all necrotic and infected tissues are removed.**
6) **amputation or loss of an extremity.**

Although immunological deficiencies in lymphocytic/macrophage cooperation and decrease in host defense cells CD-5, CD-4, CD-8, natural killer cells and CD-4/CD-8 ratios

have been shown to be implicated and associated in patients with chronic post-traumatic osteomyelitis, any definitive role in immune alteration is unclear in the etiology of chronic osteomyelitis (Peters KM, Klosterhalfen B, Zwadlo-Klarwasser G, Koberg K, Rosendahl T, Zilkens KW, Unfallchirurg 1993, 96(1):29-33; Sistermann R, Mollenhoff G, Walz M, Josten C, Muhr G, Unfallachirug 1993, 95(5):254-8). No immuno-adjuvant therapy is currently advocated in the clinical treatment of osteomyelitis.

Still another disease related to immunity is cancer. Cancer chemotherapy is associated with a fall in the number of circulating cells such as the red blood cells, the leukocytes and the platelets. Due to the property of cytotoxic drugs to kill non-malignant cells, the normal functional cells of the body are also destroyed. Thus, because of a decrease, specifically in the leukocyte number, the patients who undergo chemotherapy are especially susceptible to fulminating infection during the course of therapy. Adjuvant therapies are needed to reduce the cytotoxic chemotherapy-induced leukopenia in cancer patients.

As stated above another disease is diabetes. Diabetes Mellitus is the most common endocrine disease found among human beings. It is characterized by hyperglycemia and glycosuria and in the long term it is associated with damage, dysfunction or failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels. Several lines of evidence suggest that insulin dependent diabetes mellitus (IDDM) results from autoimmune destruction of beta cells of the pancreas leading to insulin deficiency. Lymphocytic infiltrates indicating insulitis are seen during autopsy of type-1 diabetes. Association of type-1 diabetes with polyendocrine autoimmunity and other autoimmune diseases also suggest this etiology. Now it is known that loss of insulin reserve occurs slowly over a few to many years and certain autoantibodies predate the development of the overt disease. One of the modes of therapy is initiating immunosuppressive therapy at the time of diagnosis of IDDM which can prolong the patient's ability to secrete insulin, as determined by plasma C-peptide responses to a standard mixed meal or glucagon. This beneficial effect, whether achieved by Azathioprine, Cyclosporine or anti CD5 antibodies, is not sustained in most patients. The potential side effects of immunosuppressive agents, however, have precluded their use in large trials of non-diabetic subjects at increased risk of IDDM. Another interesting method of intervention involves orally induced tolerance to islet cell antigen implicated as targets of autoimmunity to beta cells. The beneficial effects of such immunomodulatory therapy may result from the generation of T-lymphocytes that

secrete cytokines (such as interleukin-4, interleukin-10, and transforming growth factor beta) which in turn retard the autoimmune responses to the subject's own myelin or collagen. A second therapy that may also generate regulatory cytokines capable of diminishing the destruction of beta cells is treatment with Bacille Calmette-Guerin. Various therapies have thus been tried along with conventional insulin therapy. However, their use has been limited because of minimum efficacy and the potential side effects.

Other diseases relate to respiratory system disorders. Chronic Obstructive Pulmonary Disease (COPD) is one of the common problems affecting 10% of the population above the age of 45 years in the world. This is associated with frequent acute exacerbations and it contributes up to 25% of acute medical admissions to hospital. There is evidence to suggest that morbidity and mortality rates in COPD patients are rising and as such prompt and proper treatment of these patients is essential.

The specter of AIDS and the consequent alarming increase in the huge numbers of immune-compromised persons, and the high incidence of opportunistic infections have generated worldwide interest in the discovery of novel approaches and immunotherapy drugs to address the problems. So, too, is the case with drugs recommended for treatment of osteomyelitis, cancer, diabetes, and respiratory system disorders. All such introductions have, however, been found to be insufficiently effective, and display toxic side effects. There is thus a need for newer, effective and safer approaches and drugs. Recourse is being had to alternate systems of medicine like Ayurveda to find herbal remedies that could provide immunoadjuvant therapies to conventional therapy that would raise the immune status of a patient to cope with the incurred disease.

Tinospora cordifolia (Menispermaceae), also known as guduchi, is named amrita in Ayurveda and is used since ancient times for a variety of disorders. It belongs to the group of Rasayana and is found to be used in combination with other ayurvedic plants for the treatment of conditions associated with immunosuppression. In the prior art, laid-open application WO 91/08750 discloses the potential use of the cell contents of *Tinospora cordifolia* for the treatment of cancerous diseases. Only minimal clinical data is, however, provided for its use in cervix carcinoma. US Patent No. 5,529,778 describes an ayurvedic composition for the prophylaxis and treatment of AIDS, flu, tuberculosis and other immunodeficiency conditions in which composition of eight plant ingredients, one is

a water extract of *Tinospora cordifolia*. The patent, however, discloses no way of preparing a standardized aqueous extract of *Tinospora cordifolia*, and discloses no indication whatever of the specific role or advantage, if any, of *Tinospora cordifolia* over the other plants in the composition. Indian Patent No. 183805 discloses a process for the preparation of an immunomodulator from the ayurvedic medicinal plant gulvel (*Tinospora sp.*), wherein the active principle is claimed to be a polysaccharide.

A product named Adbac is said to be commercially available in India as a natural immunostimulant in the form of capsules, reported to contain 300 mg of standardized aqueous extract of guduchi (*Tinospora cordifolia*). There is no indication in the published literature, however, of the manner by which the product is standardized.

A second product named Immumod is also known to be commercially available in India with indications for use in conditions associated with non-specific suppression of immunity. Immumod is available as tablets of 100 mg / 500 mg and as a syrup. Immumod is claimed to contain an aqueous extract of *Tinospora cordifolia*.

Standardized herbal products are the bane of the herbal health care industry. Herbal products are generally mixtures of several plants. Even when such products are of single plant constituents, there is usually no knowledge of the nature of the active ingredient(s) and of the amount required of the active ingredient in the extract for the product to be effective. Plant ingredients are known to vary depending on the strain of the plant used, the nature of the soil in which the plant grows, the age of the plant, the time of harvest and related factors. There is a great need, therefore, for herbal products to be standardized by methods that quantitate one or more of its ingredients to ensure that there is continuity of quality from one extract of the plant to another. Such a standardization enables that medical or nutritional treatment regimens of disorders and deficiencies be prescribed based on quantitative norms.

Brief Description of the Figures and Tables

FIG. 1 illustrates the LC-MS SIR (Single Ion Recording) assay of a typical *Tinospora cordifolia* extract. The lowest trace depicts the fingerprint total ion chromatogram (TIC) of the methanol-soluble portion of the extract. The middle trace depicts the extracted mass chromatogram of selected ion $(M+H)^+$ equal to 342 corresponding to the extract constituent of m/z 341 mass units. The upper trace depicts the extracted mass chromatogram of selected ion $(M+H)^+$ equal to 481 corresponding to the extract constituent of m/z 480 mass units.

Detailed Description of the Invention

The inventors of the present invention have conducted an extensive study to identify the therapy that can be used alone or in conjunction with conventional therapy, and found as a result that a herbal product is suitable as a therapy alone or as an adjuvant therapy to conventional antibiotic therapy, chemotherapeutic therapy and surgical therapy in effecting a bacteriological, clinical and radiologic cure in cases of health deficiencies associated with suppression of immunity, and in particular osteomyelitis, especially chronic osteomyelitis, cancer, diabetes and respiratory disorders.

That is, the present invention relates in particular to an immunomodulating agent, and its use in therapy alone or in adjuvant therapy.

The herbal-containing immunomodulatory agent according to the present invention is found by the present inventors to be effective as adjuvant therapy to conventional cancer chemotherapy in demonstrating clinical efficacy by assessment of the decrease in the incidence of leukopenia in patients, especially breast cancer patients, on cancer chemotherapy.

The herbal-containing immunomodulating agent according to the present invention is also found by the present inventors to be effective as adjuvant therapy to conventional insulin therapy in demonstrating clinical efficacy by assessment of increase in the insulin secretory capacity, and the daily insulin requirement. It is well tolerated.

The herbal-containing immunomodulating agent according to the present invention is also found by the present inventors to be effective as adjuvant therapy to conventional antibiotic therapy and respiratory disorder amelioration therapy in demonstrating clinical efficacy in chronic bronchitis patients by assessment of the number of acute exacerbations, the forced expiratory volume and the peak expiratory flow.

The herbal-containing immunomodulating agent according to the present invention is found to be effective as adjuvant therapy to conventional antibiotic therapy in demonstrating clinical efficacy in chronic osteomyelitis by assessment of clinical parameters such as pain, tenderness, discharge, edema, itching, odor, in judging bacteriological cure as eradication or persistence of the initial causative pathogen in the post-treatment bacteriological examination, and in assessing radiological cure. It is well tolerated.

The immunomodulating agent of the invention is a novel herbal extract prepared from the plant *Tinospora cordifolia,* which is standardized on the basis of its immunomodulatory activity as measured by its potential to increase phagocytosis by polymorphonuclear leukocytes by a value of not less than 20% over a base value, and on the basis of its constituents, one of which has a mass spectrometric M+ value of m/z 480 mass units and is present to an extent of not less than 35% of the two identified peak areas of the liquid chromatography-mass spectrometry single ion recording (LC-MS SIR) chromatogram, and the second of which has a mass spectrometric M+ value of m/z 341 mass units and is present to an extent of not more than 65% of the two, identified peak areas of the LC-MS SIR chromatogram of the methanol soluble content in the said extract. The process for the preparation of an extract of the plant *Tinospora cordifolia* comprises determining, by the technique of liquid chromatography-mass spectrometry (LC-MS), and establishing a range within which the content in the said extract must lie of one of its constituents having an M^+ value of m/z 480 mass units, and of a second of its constituents having an M^+ value of m/z 341 mass units, and determining and establishing a limit and range for a phagocytosis index within which the said extract must lie. No extract of *Tinospora cordifolia* has been previously described which has been quantitatively standardized in the manner described herein. The invention has become possible because of the in-depth studies of analysis of different extracts of *Tinospora cordifolia* that the inventors have conducted by the techniques of phagocytosis by polymorphonuclear (PMN)

leukocytes, and of liquid chromatography spectrometry (LC-MS), and the identification of finger-print patterns of immunomodulatory active extracts through these techniques. The use of the technique of phagocytosis measurement by PMN leukocytes as a measure of the immunomodulatory potential of the extract of the invention does not preclude or exclude the use of other methods to evaluate the immunomodulatory potential of the extract. Such methods are known to those skilled in the art and include the carbon clearance assay in rats (Wagner et al., Plant, Med., (3), 184, 1986), Jerne's spleen plaque assay (Science, 140, 405, 1963) or the uptake of tritiated thymidine by mouse spleen cells (Indian Patent No. 183805).

Therefore, a further embodiment of the invention provides a pharmaceutical composition which comprises the standardized extract of *Tinospora cordifolia* of the invention and a pharmaceutically acceptable carrier.

The pharmaceutical compositions of this invention may be administered in standard manner for the disease condition that it is desired to treat, for example by oral, rectal or parenteral administration. For these purposes, the extracts of the invention may be formulated by means known in the art into the form of, for example, tablets, capsules, aqueous or oily solutions or suspensions, emulsions, dispersible powders, suppositories, ointments, creams, eye drops, nasal drops and sterile injections, aqueous or oily solutions or suspensions.

The extract is made available in the form of tablets and as a syrup, for oral administration. The dosage of the immunomodulating agent of the invention is appropriately selected according to the age, sex or other conditions or symptoms of a patient. Usually, a preferred dose of the agent is 1 to 50 mg/kg body weight in 3 or 4 divided doses per day for a period of 5 to 7 weeks. General recommendation for prescribing physicians is (a) for adults a tablet of 500 mg three times a day for a minimum of 15 days, (b) for children a tablet of 100 mg three times a day for a minimum of 15 days and (c) for children, aged 6 months to two years: 1/2 teaspoonful 3 times daily; aged 2-6 years: 1 teaspoonful 3 times daily; aged 7-12 years: 2 teaspoonful 3 times daily or directed by the physician. Formulations known to those skilled in the art other than the above-mentioned tablets and syrup are also encompassed in the scope of this invention.

LC-MS SIR Assay of *Tinospora cordifolia* Extract

A number of extracts of *Tinospora cordifolia* of the invention prepared according to the standardized process of the invention as hereinbelow described was subjected to LC-MS assay. The details of the LC-MS assay method developed by the inventors is described in the experimental section. FIG. 1 displays in its lowest panel a typical total ion chromatogram (TIC) shown by the extracts of the invention.

The immunomodulatory activity of the extract is measured by determining a percentage increase in phagocytosis by PMN leukocytes over a base value according to the modified method of Lehrer (Lehrer et al., Blood 1968, 32, 423-35) - cf. experimental section. All active extracts of the invention have a percentage increase of phagocytosis by PMN leukocytes of a value not less than 20% over a base value.

The stems and above-ground parts of the plant *Tinospora cordifolia* are used for preparation of the aqueous extract. The process for the preparation of an extract of *Tinospora cordifolia* comprises treating the pulverised dried aboveground parts of the plant, *Tinospora cordifolia*, into a vessel with sufficient water to soak the plant material, the temperature is raised to the boiling point, preferably by the passage of steam, for a period of about 1.5 hours to 2.5 hours, and the aqueous extract drained off. This operation of addition of water, boiling and draining is repeated two more times. The collective extract obtained is concentrated under vacuum till the concentrate analyses for a content of 20% of total solids, cooled to room temperature and filtered. The filtrate is concentrated to a thick paste analysing for a content of 60-70% total solids. The thick paste is subjected to drying preferably in a vacuum drier at 50-60°C till the dry material has a moisture content of less than 10%. In a preferred embodiment, the dried material is collected, pulverised in a mill, sieved over # 20 sieve, and checked that it passes the pharmacopeal microbial limits. In the event the material does not meet the limits of the specification for microbial counts, the pulverised powder is treated in a reactor with aqueous alcohol, preferably 50% aqueous alcohol, filtered and dried again in a vacuum drier at 50-60°C till the drug material has a moisture content less than 10% and assayed to ensure that it meets the pharmacopeal microbial limits.

The water used for the extraction of the plant material may be subjected to sterilisation by one of different techniques known to those skilled in the art, viz. exposure to ultraviolet radiation, use of millipore filters, autoclaving, and preferably by exposure to UV light of wavelength 250-261 nm for varied periods of time dependent on the quality of the water. Preferably, the aqueous extract is concentrated under vacuum at temperatures of 50 to 60°C.

As stated above, the extract is evaluated for bioactivity by evaluating the percentage increase in phagocytosis by PMN leukocytes over a base value as described in the examples. An extract passes as an active extract when the percentage increase in phagocytosis is not less than 20 % over a base value. The methanol soluble portion of the extract is subjected to LC-MS assay. The quantitative range in which the peak M+ m/z 480 mass units and the peak M+ m/z 341 mass units lie is determined. An active extract displays a percentage increase in phagocytosis of not less than 20% over a base value, and contains the peak corresponding to M+ m/z 480 mass units to an extent of not less than 35% of the two identified peak areas of the chromatogram, and also contains the second peak corresponding to M+ m/z 341 mass units to an extent of not more than 65% of the two identified peak areas of the chromatogram of the methanol soluble content of the extract.

In addition to the extract of the present invention the pharmaceutical composition of this invention may also contain, or be co-administered with, one or more known drugs selected from other clinically useful agents, in particular antibacterial agents, cancer chemotherapeutic agents, antidiabetic aagents, agents for treatment of bronchial diseases. The antibacterial agents may include penicillins, cephalosporins, fluoroquinolones, macrolides, carbapenems, the cancer chemotherapeutic agents may include cyclophosphamide, methotrexate, 5-fluorouracil, the antidiabetic agents may include insulin , the agents for treatment of bronchial diseases may include theophylline and asthalin, all such agents being agents normally used in conventional therapy with which it is desired to have the immuno-adjuvant therapy of the invention done in conjunction. A suitable pharmaceutical composition of this invention is one suitable for oral administration in unit dosage form, for example, a tablet or capsule which contains between 50 mg to 700 mg. of the extract of the invention.

In another aspect a pharmaceutical composition of the invention is one suitable as a liquid oral dosage form, such as a syrup. Yet another embodiment of the invention is the use of the standardized extract of *Tinospora cordifolia* of the invention and compositions thereof as adjuvant therapy in conjunction with conventional therapy for the treatment of different diseases due to immunodeficiency conditions, and as a supplement in food and nutritional products. Our currently copending US application entitled: Novel Method of Treatment of Health Deficiencies Associated with Immunity Suppression discloses in particular a novel method of treatment of osteomyelitis. The product and compositions of the invention can also be used for treatment of infectious diseases as tuberculosis, lower respiratory tract infections, chronic obstructive pulmonary disease, tonsilitis, otitis media, hepatitis, cancer, AIDS, diabetes mellitus, diabetic ulcers, burns, pediatric diseases. The dosage of the immumomodulating agent of the invention is appropriately selected according to the age, sex or other conditions of a patient, the symptoms, etc. Usually, a preferred dose of the agent is 1 to 50 mg/kg body weight in 3 or 4 divided doses per day for a period of 5 to 7 weeks.

The invention is illustrated, but not limited, by the following methods and examples.

Method 1 : Determination of Percentage Increase of Phagocytosis by PMN leukocytes

Polymorphonuclear (PMN) leukocytes phagocytosis assay was performed by a modified method of Lehrer (Ref. Lehrer et. al., Blood 1968, 32, 423-35). Number of PMNs: 2 x 10^6/ml; Test organisms (No.): *Candida albicans* (1 x 10^6/ml); Concentration of test drug: 0.4 mg/ml

Method 2: LC-MS SIR assay

Chemicals and reagents

Ammonium acetate used was of analytical reagent grade. HPLC grade methanol, acetonitrile and double distilled water passed through Mill-Q water purification system were used throughout the experiment.

Instrumentation

A Hewlett Packard HPLC (HP 1100) consisting of vacuum degasser, quarternary pump, autoinjector, thermostatted column compartment and variable wavelength UV detector was used. The chromatographic system consists of YMC-Pack-CN (250 x 4.6 mm, 5

micron, 120 Å) column and mobile phase (50 mM ammonium acetate and acetonitrile in gradient fashion) delivered at 1.0 ml/min. The thermostatted column compartment was maintained at 25°C. A gradient program was utilised ranging over 30 min with eluent percentages of acetonitrile increasing from 16 % to 60% and reverting to 16%.

The autoinjector was set up to make a 20 microliter injection with needle wash after each injection. The eluent from the column was split (3:1) using Valco splitter, the 75% eluent diverted to the UV detector and the 25% eluent to the electrospray probe of the mass spectrometer. Mass spectrometric determination was performed on Micromass Quattro-II, a triple quadrupole mass spectrometer operating in positive ion electrospray mode. The source temperature and desolvation temperature was 120°C and 300°C respectively. Nitrogen was used as drying gas and electrospray nebulising gas at the flow of 300 lit./hr. and 15 lit./hr. The ESI capillary potential was set at 4.0 kV and cone voltage was 30V. The LC-UV data was acquired at 240 nm. The LC-MS data was acquired from 150 to 700 Da with scan time of 1.3 sec and inter-scan delay 0.13 sec. Mass calibration and data acquisition were performed by using Windows NT based Masslynx 3.2 software. Peak areas of the UV chromatogram corresponding to m/z 341 mass units and to m/z 480 mass units were obtained by peak integration.

Sample Preparation

Anhydrous extract powder (ca. 1 gm), prepared according to the process of the invention, was transferred to a 100 ml standard volumetric flask, and dissolved in methanol (100 ml) with the use of sonication and shaking. About 50 ml was transferred to a centrifuge tube and centrifuged at 8000 rpm for 10 min. 20 ml of supernatant clear liquid was evaporated to dryness, 5 ml water was added to the residue, and the mixture was sonicated for 10 min. The mixture was filtered and passed through a previously conditioned SEP-PAK C-18 cartridge with 20 ml methanol followed by 20 ml water. The cartridge was rinsed with 10 ml water, and the retained components were eluted with 8 ml 50% aqueous methanol. The final volume was adjusted to 10 ml with aqueous methanol before the solution was used for LC-MS SIR assay.

TABLE 1

Percentage increase in phagocytosis by PMN leukocytes over a base value

Batch No. of the invention	% Increase in phagocytosis by PMN leukocytes over a base value	% peak area of M^+ (m/z 480)	% peak area of M^+ (m/z 341)
1	38.7	73.13	26.87
2	32.0	79.14	20.86
3	37.4	59.16	40.84
4	40.0	49.08	50.92
5	38.1	61.83	38.17

EXAMPLE 1: Process for making a standardized extract of *Tinospora cordifolia.*

Pulverised *Tinospora cordifolia* plant material (1 kg) is charged into a wooden vessel. UV sterilised water (2.5 lit. or sufficient quantity to soak the material) is added into the vessel and boiled with the help of steam (80°C) for 2 hours. Similar operation is repeated another two times. The collective extract is concentrated under vacuum to about 20% of total solids, cooled to room temperature and filtered through 400 micron filter cloth in a filter press with the aid of supercell. The filtrate is concentrated to a thick paste of 60-70% total solids. The thick paste is subjected to drying in a vacuum drier at 50-60oC till the dry material has a moisture content less than 10%. The dry flakes collected are pulverised in a mill and sieved over 20 #.

EXAMPLE 2:

The following description illustrates representative pharmaceutical dosage forms containing the extract of the invention for therapeutic or prophylactic use in humans:

(a) Tablet

Tablet 1	Mg/tablet
Extract of the invention	55.00 - 700.00
Microcrystalline cellulose	10.00 - 127.00
Lactose	11.50 - 146.00
Silicon dioxide	2.00 - 25.40

Cross carmellose sodium	0.80 - 10.20
Methyl paraben	0.14 - 1.78
Propyl paraben	0.04 - 0.51
Bronidiol	0.02 - 0.25
Magnesium stearate	0.50 - 6.35
For film coating of the tablets:	
Isopropyl alcohol	
Hydroxypropyl methyl cellulose	
Diethyl phthalate	
Methylene cloride	
Erythrocin aluminium lake	
Methylene chloride	
Erythrocin aluminum lake	
Sunset yellow aluminum lake	
Ponceau 4 R aluminum lake	
Carnauba wax	

(b) Syrup

Syrup 1	Qty/1.25 ml. - Qty/10 ml.
Extract of the invention	25.00 - 200.00 mg.
Sucrose	0.63 - 5.00 gms.
Sodium methyl paraben	1.88 - 15.00 mg.
Sodium propyl paraben	0.63 - 5.00 mg.
Bronidiol	0.25 - 2.00 mg.
Sodium saccharin	2.50 - 20.00 mg.
Liquid glucose	0.33 - 2.86 gms
Caramel	1.20 - 10.00 ml.
Flavour cardamom 21180	0.0013 - 0.01 ml.
Purified water q.s. to	1.25 - 10.00 ml.

The above formulations may be obtained by conventional procedures well known in the pharmaceutical art. The tablet (a) may be film coated and a suitable color included by conventional means.

Test Example 1:

Effect of the extract of the invention as adjuvant therapy in patients with osteomyelitis.

Extract of the invention (1 tablet, 500 mg) and matching Placebo tablets were administered twice daily for 6 weeks to a randomized group of 50 patients (36 males and 14 females) diagnosed as suffering from subacute to chronic osteomyelitis. In addition to the immunomodulating extract of the invention, all patients received antibiotic therapy in the form of Tab pefloxacin (400 mg) twice daily for 6 weeks.

After 6 weeks from the start of the treatment with the tablets, the physician in charge judged the degree of symptomatic improvement, clinical efficacy, bacteriological response and radiological assessment in the patients on the immunomodulating extract of the invention/placebo therapy based on 3 scales of cure, improvement and failure. These results are shown in Table 2.

Table 2

Symptom Score/Cure Improvement Index (I/PXI00)*

Symptom Evaluation	103
Clinical Evaluation	120
Bacteriological Response	119
Radiological Assessment	97

*I = Extract of the invention data

P= Placebo data

The results indicate the improvements seen with the immunomodulating agent in symptom evaluation, clinical evaluation and bacteriological response. Radiological improvements are known to be seen long after the drug treatment of an infective condition is completed.

It is seen from these results that the immunomodulating agent according to the present invention brings about improvement in symptoms and cure of osteomyelitis, especially chronic osteomyelitis, exhibiting effectiveness over patients treated only with conventional therapy.

Test Example 2:

Effect of extract of the invention against cytotoxic chemotherapy induced leukopenia in breast cancer patients.

The extract of the invention (1 tablet, 500 mg) and matching Placebo tablets were administered thrice daily for 14 days as per a chemotherapy cycle protocol to a randomized, double blind placebo clinical trial group of 38 patients diagnosed as suffering from breast cancer. All patients also received chemotherapy in the form of cyclophosphamide 750 mg/m^2, methotrexate 40 mg/m^2 and 5-fluorouracil 750 mg/ m^2 every 3 weeks. An absolute end point for each cycle of chemotherapy for every patient was the appearance of leukopenia (leucocyte, WBC count < 3000 mm^3). The results may be summarised as follows:

1. There was no difference in the basal WBC counts of both groups indicating that the groups were similar at the beginning
2. There was a significant leukopenia in both the groups. However, the number of patients with total WBC counts less than 3000/cu mm were significantly less ($p<0.05$, chi square test) in the group administered the extract of the invention (55%) as compared to the placebo treated group (70%).
3. There were 24 cycles in the placebo group where the count fell below 2000/cu mm while there were only 14 in the treated group.

The results indicate that treatment with the immunomodulating extract of the invention was found to decrease the incidence of leukopenia in patients, especially in breast cancer patients, on cancer chemotherapy, exhibiting such effectiveness over patients treated with only conventional chemotherapy. The conclusion suggested is that the extract of the invention (a) induces leukocytosis thus increasing the WBC counts and (b) induces the release of granulocyte macrophage - colony stimulating factor (GM-CSF), as shown in animal studies, thus abating leukopenia.

Test Example 3:

Effect of the extract of the invention on the insulin secretory reserve in Type 1 diabetic patients on Insulin therapy.

The extract of the invention (1 tablet, 500 mg) and matching Placebo tablets were administered thrice daily for 4 weeks to a randomized group of 50 patients (34 malesand 16 females) diagnosed as suffering from IDDM. The patients in the group administered the extract of the invention as well as insulin therapy were designated as cases. The patients in the other group who received only insulin therapy were designated as controls. After 4 weeks from the start of the treatment with the tablets, the physician in charge judged the degree of clinical efficacy on the basis of the following parameters:

1. **Blood glucose levels (Fasting and 2-hour post-load glucose)**
2. **Serum C-peptide levels basal, stimulated and percentage rise in the C-peptide level over the basal level 2-hours following 75 grams oral glucose load.**
3. **Glycosylated haemoglobin**
4. **Total daily insulin requirements**
5. **Quality of life/performance status**

The results of the study in respect of the insulin secretory reserve and the daily insulin requirements were as follows:

1. **The mean percentage rise in the insulin secretory reserve in the cases and the controls was 25.96 ± 15.07 and 0.19 ± 14.55 respectively which is highly statistically significant.**
2. **The mean percentage rise in the daily insulin requirements in cases was 20.17 ± 26.59 and that in controls was 84.86 ± 36.04 which is highly statistically significant.**

The results indicate the improvements seen with the immunmodulating agent in increased insulin secretory capacity and reduction in insulin requirement. It is seen from these results that the immunomodulating agent according to the present invention brings about improvement in symptoms and cure of IDDM exhibiting effectiveness over patients treated only with conventional therapy.

Test Example 4:

Effect of the extract of the invention in Chronic Bronchitis Patients.

The extract of the invention (1 tablet, 500 mg) and matching Placebo tablets were

administered thrice daily for 8 weeks to a randomised group of 60 patients. In addition to the immunomodulating extract of the invention, all acute exacerbations were treated with Roxithromycin 150 mg BD, Theophylline 200 mg BD and Asthalin rotahaler.

After 8 weeks of therapy, the physician in charge judged the degree of efficacy on the basis of the following parameters:

1. **Reduction in number of Acute Exacerbations during 8 weeks of study compared to the previous 2 months.**
2. **Improvement in Forced Expiratory volume in 1 sec measured by spirometry.**
2. **Improvement in Peak expiratory flow measured by spirometry.**

The results may be summarised as follows:

1. **Significant reduction in the episodes of Acute Exacerbations in the test group 2.06 ± 0.41 as compared to the control group 3.90 ± 0.79.**
2. **Significant improvement in the Forced Expiratory volume in the test group 42.36 ± 10.32 as compared to the control group 33.63 ± 5.73.**
3. **Significant improvement in the Peak Expiratory flow in the test group 30.70 ± 8.37 as compared to the control group 24.53 ± 4.58.**

The results indicate that the treatment with the immunomodulating extract of the invention was found to decrease the incidence of acute exacerbations in chronic bronchitis patients and to improve the forced expiratory volume and peak expiratory flow exhibiting such effectiveness over patients treated with only conventional therapy. It can be concluded that by inducing phagocytosis and release of GM-CSF, as shown in animal studies, the extract of the invention decreases the incidence of infections and acute exacerbation in patients with chronic bronchitis.

<u>Other Test Examples</u>

Similarly data can be provided for treatment of animals in pharmacological models of immunomodulatory conditions and for treatment of humans suffering from disorders and infectious diseases as tuberculosis, lower respiratory tract infections, chronic obstructive pulmonary disorders, tonsilitis, otitis media, hepatitis, cancer, AIDS, diabetes mellitus, diabetic ulcers, burns, pediatric diseases.

CLAIMS

1. A method of treatment of health deficiencies associated with modulation of immunity, through inclusion in conventional therapy of the use of adjuvant therapy, which method comprises administering a standardized herbal extract prepared from the plant *Tinospora cordifolia*, in conjunction with conventional therapy.

2. A method of treatment according to Claim 1 in which the herbal extract is standardized on the basis of its immunomodulatory activity as measured by its potential to increase phagocytosis by polymorphonuclear leukocytes by a value of not less than 20% over a base value, and on the basis of its constituents, one of which has a mass spectrometric M+ value of m/z 480 mass units and is present to an extent of not less than 35% of the two identified peak areas of the liquid chromatography mass spectrometry single ion recording (LC-MS SIR) chromatogram, and the second of which has a mass spectrometric M+ value of m/z 341 mass units and is present to an extent of not more than 65% of the two identified peak areas of the LC-MS SIR chromatogram of the methanol soluble content of the said extract.

3. A method of treatment according to Claim 1 for the immunodeficient condition of osteomyelitis.

4. A method of treatment according to Claim 1 for the immunodeficient condition of cancer.

5. A method of treatment according to Claim 4 for the immunodeficient condition of breast cancer.

6. A method of treatment according to Claim 1 for the immunodeficient condition of diabetes.

7. A method of treatment according to Claim 1 for the immunodeficient condition of respiratory tract diseases.

8. A method of treatment according to Claim 1 for the immunodeficient condition of chronic bronchitis.

9. A method of treatment according to Claim 1 for disorders and infectious diseases as tuberculosis, lower respiratory tract infections, chronic obstructive pulmonary diseases, tonsilitis, otitis media, hepatitis, cancer, AIDS, diabetes mellitus, diabetic ulcers, burns, pediatric diseases.

10. A method of treatment according to claim 1 wherein administration of treatment is systemic or topical.

11. A method of treatment according to Claim 10 wherein the systemic oral dosage form is a tablet, capsule or syrup.

12. A method of treatment according to Claim 11 wherein the daily dosage is from 25 mg to 1500 mg in divided doses for a period as recommended by the physician.

13. A process for preparation of the standardized extract of *Tinospora cordofolia* which comprises treating the plant material with water at an elevated temperature, filtering, concentrating and pulverizing to provide an extract that meets the defined standardization limits as its immunomodulatory activity as measured by its potential to increase phagocytosis by polymorphonuclear leukocytes by a value of not less than 20% over a base value, and on the basis of its constituents, one of which has a mass spectrometric M+ value of m/z 480 mass units and is present to an extent of not less than 35% of the two identified peak areas of the liquid chromatography mass spectrometry single ion recording (LC-MS SIR) chromatogram, and the second of which has a mass spectrometric M+ value of m/z 341 mass units and is present to an extent of not more than 65% of the two identified peak areas of the LC-MS SIR chromatogram of the methanol soluble content of the said extract.

AMENDED CLAIMS
[received by the International Bureau on 29 May 2002 (29.05.02);
original claims 1-13 replaced by new claims 1-31 (3 pages)]
+ STATEMENT

1. A method of treatment of a health condition associated with modulation of immunity which method comprises administering a standardized herbal extract prepared from the plant *Tinospora cordifolia*.

2. A method of treatment of a health condition associated with modulation of immunity which method comprises administering a standardized herbal extract prepared from the plant *Tinospora cordifolia* in conjunction with another treatment for the health condition.

3. A method of treatment according to claim 1, in which the herbal extract is standardized on the basis of its immunomodulatory activity as measured by its potential to increase phagocytosis by polymorphonuclear leukocytes by a value of not less than 20% over a base value, and on the basis of its constituents, one of which has a mass spectrometric M+ value of m/z 480 mass units and is present to an extent of not less than 35% of the two identified peak areas of the liquid chromatography mass spectrometry single ion recording (LC-MS SIR) chromatogram, and the second of which has a mass spectrometric M+ value of m/z 341 mass units and is present to an extent of not more than 65% of the two identified peak areas of the LC-MS SIR chromatogram of the methanol soluble content of said extract.

4. A method of treatment of a health condition associated with modulation of immunity according to claim 2, in which the herbal extract is standardized on the basis of its immunomodulatory activity as measured by its potential to increase phagocytosis by polymorphonuclear leukocytes by a value of not less than 20% over a base value, and on the basis of its constituents, one of which has a mass spectrometric M+ value of m/z 480 mass units and is present to an extent of not less than 35% of the two identified peak areas of the liquid chromatography mass spectrometry single ion recording (LC-MS SIR) chroma-togram, and the second of which has a mass spectrometric M+ value of m/z 341 mass units and is present to an extent of nor more than 65% of the two identified peak areas of the LC-MS SIR chromatogram of the methanol soluble content of said extract.

5. A method of treatment of a health condition associated with alteration or modulation of immunity which method comprises administering a standardized herbal extract prepared from the plant *Tinospora cordifolia* wherein the herbal extract is standardized on the basis of its immunomodulatory activity as measured by its potential to increase phagocytosis by polymorphonuclear leukocytes by a value of not less than 20% over a base value, and on the basis of its constituents, one of which has a mass spectrometric M+ value of m/z 480

mass units and is present to an extent of not less than 35% of the two identified peak areas of the liquid chromatography mass spectrometry single ion recording (LC-MS SIR) chromatogram, and the second of which has a mass spectrometric M+ value of m/z 341 mass units and is present to an extent of not more than 65% of the two identified peak areas of the LC-MS SIR chromatogram of the methanol soluble content of said extract in conjunction with another therapy for the health condition.

6. The method of treatment according to claim 2, wherein the condition is osteomyelitis.

7. The method of treatment according to claim 4, wherein the condition is osteomyelitis.

8. The method of treatment according to claim 5, wherein the condition is osteomyelitis.

9. The method of treatment according to claim 2, wherein the condition is cancer.

10. The method of treatment according to claim 4, wherein the condition is cancer.

11. The method of treatment according to claim 5, wherein the condition is cancer.

12. The method of treatment according to claim 2, wherein the condition is breast cancer.

13. The method of treatment according to claim 4, wherein the condition is breast cancer.

14. The method of treatment according to claim 5, wherein the condition is breast cancer.

15. The method of treatment according to claim 2, wherein the condition is type 1 diabetes.

16. The method of treatment according to claim 4, wherein the condition is type 1 diabetes.

17. The method of treatment according to claim 5, wherein the condition is type 1 diabetes.

18. The method of treatment according to claim 2, wherein the condition is tuberculosis, lower respiratory tract infections, tonsilitis, otitis media, hepatitis, cancer, AIDS, diabetes mellitus, diabetic ulcers, burns or pediatric disease.

19. The method of treatment according to claim 4, wherein the condition is tuberculosis, lower respiratory tract infections, tonsilitis, otitis media, hepatitis, cancer, AIDS, diabetes mellitus, diabetic ulcers, burns or pediatric disease.

20. The method of treatment according to claim 5 wherein the condition is tuberculosis, lower respiratory tract infections, tonsilitis, otitis media, hepatitis, cancer, AIDS, diabetes mellitus, diabetic ulcers, burns or pediatric disease.

21. The method according to claim 2, wherein the condition is a respiratory tract disease.

22. The method according to claim 4, wherein the condition is a respiratory tract disease.

23. The method according to claim 5, wherein the condition is a respiratory tract disease.

24. The method according to claim 2, wherein the condition is chronic bronchitis.

25. The method according to claim 4, wherein the condition is chronic bronchitis.

26. The method according to claim 5, wherein the condition is chronic bronchitis.

27. The method according to according to any one of claims 1 to 26, wherein the daily dosage is 1-50 mg/kg of body weight.

28. The method according to any one of claims 1 to 26, wherein the daily dosage is from 25mg to 1500 mg.

29. A process for preparation of the standardized extract of *Tinospora cordifolia* which comprises treating the plant material with water at an elevated temperature, filtering and concentrating to provide an extract that meets the defined standardization limits as its immunomodulatory activity as measured by its potential to increase phagocytosis by polymorphonuclear leukocytes by a value of not less than 20% over a base value, and has one constituent which has a mass spectrometric M+ value of m/z 480 mass units and is present to an extent of not less than 35% of the two identified peak areas of the liquid chromatography mass spectrometry single ion recording (LC-MS SIR) chromatogram, and has a second constituent which has a mass spectrometric M+ value of m/z 341 mass units and is present to an extent of not more than 65% of the two identified peak areas of the LC-MS SIR chromatogram of the methanol soluble content of said extract.

30. An extract of *Tinosporia cordifolia* prepared by the process comprising treating the pulverized above ground parts of the plant *Tinosporia cordifolia* with water at an elevated temperature, filtering and concentrating to provide an extract that has immunomodulatory activity as measured by its potential to increase phagocytosis by polymorphonuclear leukocyte by a value of not less than 20% over a base value, and has one constituent which has a mass spectrometric M+ value of m/z 480 mass units and is preset to an extent of not less than 35% of the two identified peak areas of the liquid chromatography mass spectrometry single ion recording (LC-MS SIR) chromatogram, and has a second constituent which has a mass spectrometric M+ value of m/z 341 mass units and is present to an extent of not more than 65% of the two identified peak areas of the LC-MS SIR chromatogram of the methanol soluble content of said extract.

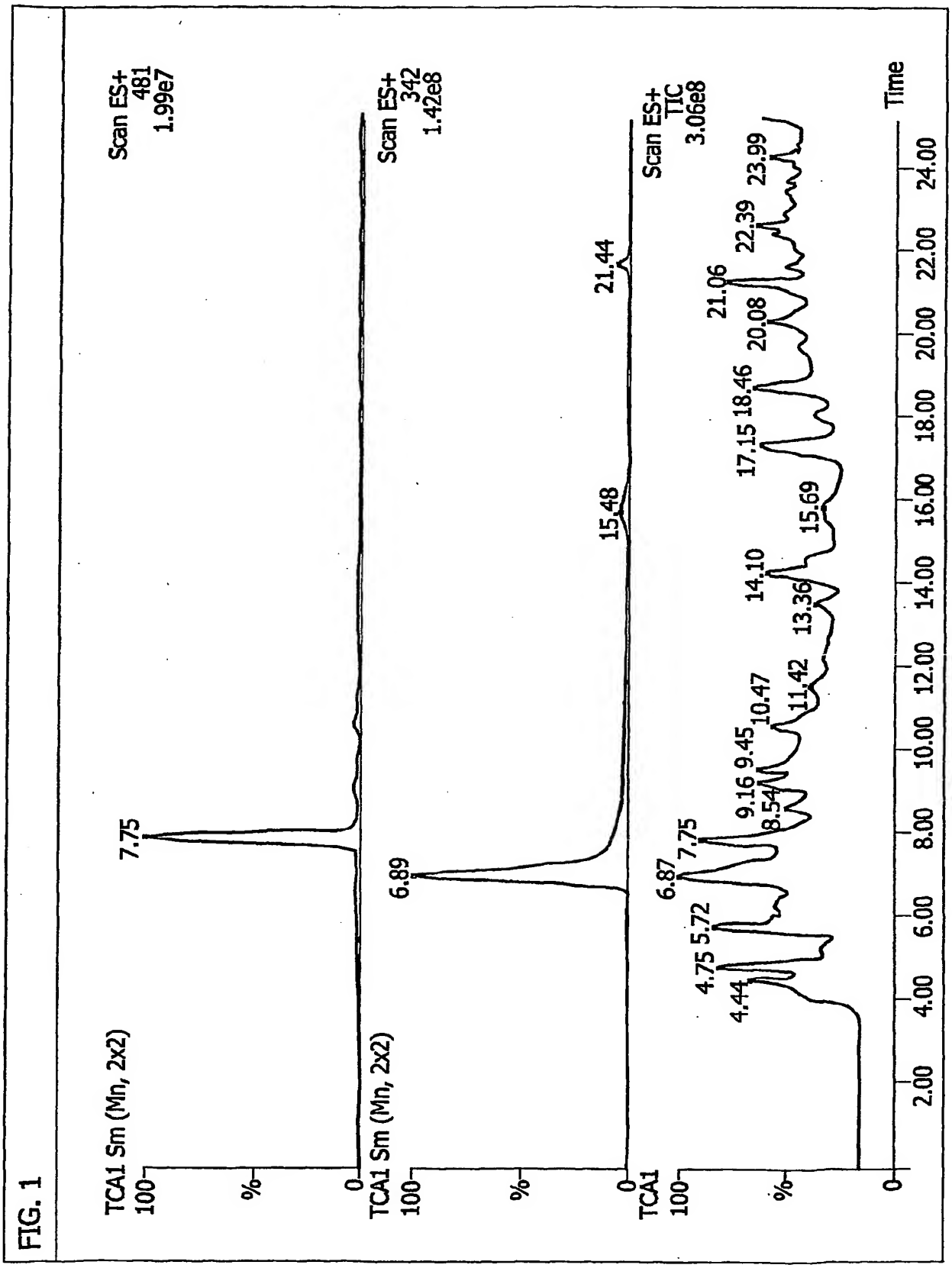
31. A composition comprising the extract of claim 30.

Statement under Article 19(1):

We hereby declare that these amendments do not go beyond the disclosure in the international application as filed and also these amendments have no impact on the description and the drawings of the specification originally filed.

NDD/PCT/009 1/1

FIG. 1



INTERNATIONAL SEARCH REPORT

PCT/IN 01/00225

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K35/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	G. CHINTALWAR ET AL: "An immunologically active arabinogalactan from Tinospora cordifolia" PHYTOCHEMISTRY, vol. 52, 1999, pages 1089-1093, XP004291119 page 1091; figure 1 page 1089, right-hand column, paragraph 2. -page 1090, right-hand column	1,2,13
X	A. KAPIL ET AL: "Immunopotentiating compounds from Tinospora cordifolia" JOURNAL OF ETHNOPHARMACOLOGY, vol. 58, 1997, pages 89-95, XP002196028 abstract page 93, left-hand column, paragraph 4. -page 94, right-hand column	1,2,4,5, 9,10

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 April 2002	26/04/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Siatou, E

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/IN 01/00225

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	THATTE UM ET AL: "Tinospora Cordifolia Induces Colony Stimulating Activity in Serum" JOURNAL OF POSTGRADUATE MEDICINE, 'Online! vol. 40, no. 4, 1994, XP002196029 Retrieved from the Internet: <URL:http://www.ncbi.nlm.nih.gov/entrez/query.fcgi> 'retrieved on 2002-04-12! abstract	1,2
X	J.N. DHULEY: "Effect of some Indian herbs on macrophage functions in ochratoxin A treated mice" JOUNAL OF ETHNOPHARMACOLOGY, vol. 58, 1997, pages 15-20, XP002196030 abstract page 19, left-hand column, line 27 - line 36	1,2
X	DATABASE PUBMED 'Online! "Immunotherapy with Tinospora Cordifolia: a new lead in the management of obstructive jaundice" retrieved from HTTP://WWW.NCBI.NLM.NIH.GOV/ENTREZ/QUERY.FCGI XP002196032 & Indian J. Gastroenterol. 1993 Jan; 12(1), 5-8 (N. Rege et al) abstract	1,2
X	DATABASE PUBMED 'Online! "Modulation of immunosuppresion in obstructive jaundice by tinospora cordifolia" retrieved from HTTP://WWW.NCBI.NLM.NIH.GOV/ENTREZ/QUERY.FCGI XP002196033 &Indian J Med Res, Dec 1989, 90:478-83 (NN Rege et al) abstract	1,2
A	G. C. JAGETIA ET AL: "Evaluation of the antineoplastic activity of guduchi (Tinospora cordifolia) in cultured HeLa cells" CANCER LETTERS, vol. 127, 1998, pages 71-82, XP002196031 abstract page 72, right-hand column, paragraph 2.1	1,2,4,5

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INTERNATIONAL SEARCH REPORT

Int onal Application No
PCT/IN 01/00225

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 08750 A (C. KRÜGER) 27 June 1991 (1991-06-27) cited in the application the whole document	1-13

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir ional Application No
PCT/IN 01/00225

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9108750 A	27-06-1991	AU 6746990 A	18-07-1991
		WO 9108750 A1	27-06-1991